



Attorneys at Law

Suite 2800 1100 Peachtree St.
Atlanta GA 30309-4530
t 404 815 6500 f 404 815 6555
www.KilpatrickStockton.com

December 18, 2003

direct dial 404 745 2408
direct fax 404 541 3363
RRichards@KilpatrickStockton.com

FAX

RECIPIENT/ PHONE NO.	FAX NO.	COMPANY/ CITY, STATE, COUNTRY
Examiner Badio	(703) 746-5003	U.S. Patent & Trademark Office

Robert E. Richards

FROM

18

PAGES (WITH COVER)

05213-0494

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CLIENT/MATTER NO.

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COMMENTS

Inventor: D'AMATO ET AL.

Serial No. 09/780,650

Filed: February 12, 2001

For: ESTROGENIC COMPOUNDS AS ANTI-MITOTIC AGENTS

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#22
n. n.
9/30/04

Patents

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

D'AMATO ET AL.

Serial No.: 09/780,650

Filed: February 12, 2001

For: ESTROGENIC COMPOUNDS AS
ANTI-MITOTIC AGENTS

Examiner: B. Badio, Ph.D.

Art Unit: 1616

DECLARATION OF ROBERT J. D'AMATO
UNDER 37 CFR §1.131

Robert J. D'Amato declares as follows:

1. I reside at Lexington, Massachusetts 02420, and am one of the inventors named in the above-referenced application for Letters Patent.
2. Attached hereto as Exhibit 1 are true and correct copies of several pages from my laboratory notebook. The dates of these pages have been redacted from the copies attached hereto. These notebook pages show tests conducted by me and demonstrate the ability of 2-methoxyestradiol to inhibit microtubule formation. All of the notebook pages comprising Exhibit 1 are dated prior to July 2, 1993.
3. Attached hereto as Exhibit 2 is a true and correct copy of a letter from me to Dr. Hamel with the National Cancer Institute. The date of this letter has been redacted from the copy attached hereto. The letter states as follows:

This letter serves to formalize our conversation regarding the estrogen metabolite 2 methoxy estradiol which we have discovered is an effective chemotherapeutic agent and microtubule inhibitor.

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office (Fax No. 703/46-5003) on the date shown below.

Typed Name of Person Signing Certification

Signature

December 18, 2003

Date

This letter (Exhibit 2) was written by me and is dated prior to July 2, 1993. This document has also been redacted to remove non-relevant material.

4. Attached hereto as Exhibit 3 is a true and correct copy of a letter from Christopher Dippel of Harvard Medical School to Mr. Takeda. The date of this letter has been redacted from the copy attached hereto. This letter states as follows:

At the meeting, Dr. Folkman described the identification of previously unreported antiangiogenic properties of an known urinary metabolite of steroid metabolism, 2-methoxy-estradiol (2-MO). ***

Upon returning to Boston, Dr. Folkman asked a postdoctoral fellow, Dr. Robert D'Amato to initiate the screening of 2-MOE for activity. Dr. D'Amato found that 2-MOE inhibits growth of vessels in the cam assay but not in the rabbit eye assay. In addition, he tested the compound in mouse against Lewis lung carcinoma and estimated a T/C of .35. In culture, 2-MOE was equally effective in preventing the growth of Lewis lung and endothelial cells, which suggests to Dr. D'Amato that 2-MOE is an antimitotic agent. Additionally, he has demonstrated that it inhibits microtubule formation similar to vinblastine, a current anticancer agent. When tested in vitro with AGM-1470, 2-MOE's effect was slightly increased.

This letter (Exhibit 3) was written by Mr. Dippel and is dated prior to July 2, 1993. This document has also been redacted to remove non-relevant material.

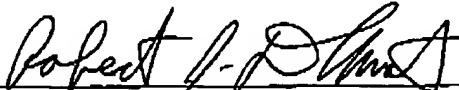
5. The foregoing documents clearly demonstrate that Dr. Folkman and I conceived of and actually reduced to practice the invention claimed in the above-referenced patent application prior to July 2, 1993.

6. The undersigned declares further that all statements made herein of his own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements, and the like so made, are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing on this application.

Date:

12/17/03

Robert J. D'Amato



Serial No. 09/780,650

- 2 -

ATLL/B02 146249.1

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Test 2-methoxy estradiol (50 mg)

(1, 3, 5[10] Estratrien - 2, 3, 17 β Triol 2-methyl ether)

Try 100 microgram/10ul on 6 eggs

100 microgram/10ul

+ 50 microgram/10ul Baybation tetradecanoic

6 eggs

1, 3, 5(10) Estratrien-2, 3, 17 β -Triol-2-methyl Ether

Sample I Estradiol alone at 100 μ g/10ul in 0.45% methylcellulose

Sample II Estradiol ~~alone~~ at 100 μ g/10ul + BCD at 50 μ g/10ul
(A0698-047-Q1)

6 am Saturday Aug 1 = 38-40 hours

Estradiol 100 μ g alone	(+)	(+)	(+)	
	nice anascula zona (still some crystals)			
Estradiol 100 μ g + BCD 50 μ g/10ul	(+)	(+)		
	still some crystals			

1st steroid I have seen that inhibits by itself. Does it have ~~an~~ estrogenic activity?

British Biotech #94

Glass beads added to sample of 8.4mg - 168ul for
500ug/10ul

10ul Tween 80^{plus} = smooth suspension

30 am Saturday Aug 1 = 38 hours

3

BB94 500ug/10ul	Early polygons		
BB94 250ug/10ul	⊖		

BB94

96 hours - m rdy

500 = ⊕ ⊖ ⊕ ⊕ ⊖ ⊕ ⊕

250 ⊕ ⊕

9³⁰ am Monday Aug 3, = 96 hours - Estimated

⊕ ⊕ ⊕ -
crystals still in

Dead

||

⊕ ⊕ ⊖ ⊕
crystals
still present

|||

~~Does Not need hyperin or exfoliation~~

2-methoxy-estradiol continued to produce nice

⊕ / vascular zones in all eggs up to 6 days after
implantation - zones still in on Day 7
this is most potent and has been important

For Lewis Levy - Jeff Grossfeld = AGM170

BB94 suspended in PBS + 0.01% Tween80 (v/v)
at 2.5 mgm/ml + glass beads - shake overnight

Give each 20 gram mouse 0.24 ml (up) daily

$$= \boxed{30 \text{ mgm/Kg}}$$

$$= 25 \text{ mgm} / 10 \text{ ml}$$

$$= 50 \text{ mgm} / 20 \text{ ml}$$

$$= 100 \text{ mgm} = 40 \text{ ml}$$

2-methoxy-estradiol suspended in PBS + 0.01% Tween80

2 ml contains 50 milligrams + 0.2 methyl

$$= 25 \text{ mgm/ml}$$

2.5 mgm / 0.1 ml per 20 gram mouse
S.C. / day

$$= \boxed{125 \text{ mg/Kg}}$$

I put 150 mgm 2-methoxy-estradiol
(Sterabids) into 6 ml 0.45% methylcellulose

in Ringers - Striched thru Nalgene filter.
~~Smashed~~ Smashed large crystals & spatula.

Added next stemmed glass beads.

No methylcellulose Tween80

Revised in cold room overnight

$$= 2.5 \text{ mgm} / 0.1 \text{ ml}$$

$$0.1 \text{ ml} = 125 \text{ mg/Kg}$$

EXHIBIT

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EN000348

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3 Methoxy estradiol

200

- ① small zone
- ② small, avascular zone
- ③ - floated off
- ④ - died

100

- ① small avascular zone
- ② small avascular zone
- ③
- ④

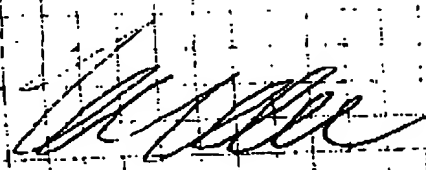
EM 12

400

- ①
 - ②
 - ③
 - ④
- > floated off

200

- ①
 - ②
 - ③
 - ④
- > floated off



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Estradiol

200 $\left\{ \begin{array}{l} \textcircled{1} \oplus \\ \textcircled{2} \ominus \\ \textcircled{3} \ominus \end{array} \right.$ floated off
100 $\left\{ \begin{array}{l} \textcircled{1} \ominus \\ \textcircled{2} \oplus \\ \textcircled{3} \ominus \end{array} \right.$ floated

EM 12

200 - floated
100 - floated

4 Hydroxy tamoxifen

200 $\left\{ \begin{array}{l} 2 \text{ died} \\ \textcircled{+} \end{array} \right.$
100 $\left\{ \begin{array}{l} 2 \text{ die} \\ \textcircled{-} \end{array} \right.$

140

(All components in suspension)

① 3 methoxy 2methoxy estradiol

200 — ① float
 ② float
 ③ died
 ④ float
 100 — ① faint effect
 ② no effect
 ③ died
 ④ died
 50 — ① floated
 ② no effect
 ③ died
 ④ no effect

② 4 methoxy estradiol

200 — ① floated
 ② floated
 ③ no effect
 ④ no effect
 100 — ① floated
 ② floated
 ③ no effect
 ④ no effect

③ estrone

200 — all

④ 4 methoxy estiol

200 — ① died
 ② died
 ③ good zone but edges of egg had mold
 ④ died
 100 — ① died
 ② died
 ③ died
 ④ died

⑤ 3 carboxymethyl estradiol

200 — ① died
 ② died
 ③ died
 ④ floated
 100 — ① died
 ② faint effect
 ③ died
 ④ floated

⑥ 17 acetate estradiol

200 — ① faint effect
 ② floated
 ③ floated
 100 — ① floated
 ② floated
 ③ floated

⑦ EM 12

400 — ① ±/± ←
 ② floated
 200 — ① floated
 ② floated
 ③ floated

⑧ 4 Hydroxy tamoxifen

200 — ① floated
 ② floated
 ③ floated
 100 — ① floated
 ② floated
 ③ floated

Dr. D'Amato

- ① 3 Methoxy-2-Methoxy Estradiol 200 μ g and 100 μ g/10ml
 ② 4 Methoxy-estradiol - 200 μ g/10ml and 100 μ g/10ml
 ③ Carbonylmethyl-estradiol - 200 μ g/10ml and 100 μ g/10ml
 ④ EM12 - 400 μ g/10ml and 200 μ g/10ml
 ⑤ Colchicine - 100 μ g/10ml
 200 Not done, to Brittle to handle
 ⑥ 2 methoxy estrone - 200, 100, and 50 μ g/10ml

All insoluble, very hard to handle, many floated
 to Complete Aug 24th # 5, 6, 7

200	- died floated faint affect	100 μ g	died died pellet broken part ⊕
200	- / pellet broken died faint	100 μ g	⊕ died ⊕
200	Floated ⊕ died died	100 μ g	- / faint died ⊕
400	- died floated	200 μ g	- floated died floated
200	NOT done Too Brittle	100	
200	① - ② died ③ died ④ white spot (scar) + avascular zone	100	① died ② floated ③ floated ④ small white spot with partial avascular zone
50	- died floated floated		

J. Folkm¹⁴⁸

make new batch 2-methoxyestradiol
for Lewis Lung mice receiving AEM-1470

- ① To achieve dose of 12.5 mgm/Kg mouse,
- ② Each 20 gram mouse must receive 2.5 mgm
- ③ If each 0.1 ml contains 12.5 mgm,
- ④ Then ~~a~~ a daily injection ~~with~~ of 0.2 ml will administer 2.5 mgm

⑤ ~~To make~~

$$1.25 \text{ mg}/0.1 \text{ ml} = 12.5 \text{ mg}/1 \text{ ml}$$

⑥ So add 75 mgm to 6 ml = 12.5 mg/ml

⑦ Use old Ringers 0.45% w/v methylcellulose (1989)
and mixed = Fresh Ringers 1:1 to
make 0.22% methylcellulose

Put thru millipore

⑧ added heat sterilized glass beads

⑨ add 2ul Tween 80

⑩ Put on shaker in old cold room.
at beginning - looks like fine particles
but after 24 hrs = like slurry



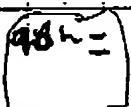




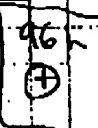





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Dr. D'Amato

4 samples each concentration \rightarrow 200 $\mu\text{g}/10\text{ml}$
100 $\mu\text{g}/10\text{ml}$

- ① Estrone-3-Methyl ether
- ② Estriol-3-methyl ether
- ③ 2-Methoxy estradiol
- ④ Bolchicine 25 $\mu\text{g}/10\text{ml}$ and 5 μg
- ⑤ β -Estradiol Benzate
- ⑥ 17 α -Ethinyl estradiol
- ⑦ 17 α -Ethinyl estradiol 3-Methyl Ether

#6 and #7 pieces - hard to handle

	200 $\mu\text{g}/10\text{ml}$	100 $\mu\text{g}/10\text{ml}$	
①	\pm Floated died	\pm Floated	
②	\pm Floated	\pm	
③	\pm \oplus/\ominus  	\pm 	
④	25 $\mu\text{g}/10\text{ml}$ died	5 $\mu\text{g}/10\text{ml}$ died	1 μg died
⑤	\pm \pm \pm  	faint (but may improve) \pm \pm 	
⑥	\oplus/\ominus \oplus/\ominus Floated died	 	 
⑦	\pm \pm died		

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Vinblastin And Methyl Estradiol
on
12 Day Rams

Vinblastin concentration 0.025 μ g / 10 μ l

Methopyle² (Methoxy Estradiol)

2mo - 100 μ g / 10 μ l

zones of
disrupted B1

2mo

⊕
⊕
⊕

small zones
but real.

Feb 10 Amata

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1. 1,3,5(10) Estratriene - 2,3,17 triol, 2,3, Dimethyl Ether

200, 100, + 50
2. 1,3,5(10) Estratrien 3,4,17, triol dimethyl ether

3. 1,3,5(10) Estratrien 2,3,17b 3 methyl ether

4. 2MOE 200 μ g or 100 μ g

2 and # 3 at lower
Concentration could not find disc
50 μ g, 25 μ g and 10 μ g

#1 200	++ 0 DD --	#3 50	—
100	+++ D	#3 25	faint
50	++ +++	#3 10	lots of crystals (-)
#2 200	++ ++	#4 200	DD ⊕ ⊕ ⊕ F starts in cap
100	++ ++ 0	#4 100	+/- +/-
50	++ ++ 0		
#3 200	++ F D F		
100	++ ⊕ +/-		

HARVARD MEDICAL SCHOOL

DEPARTMENT OF SURGERY



THE CHILDREN'S HOSPITAL
300 LONGWOOD AVENUE
BOSTON, MASSACHUSETTS 02115
(617) 735-7496 or 735-7497

Dr. Hamel
Building 37, Room 5c
NIH/NCI
Bethesda, MD 20892

Dear Dr. Hamel,

This letter serves to formalize our conversation regarding the estrogen metabolite 2 methoxy estradiol which we have discovered is an effective chemotherapeutic agent and microtubule inhibitor.

Sincerely,

Robert D'Amato M.D. Ph.D.

Dept Surgical Research
Enders 10
Children's Hospital
Boston, Mass 02115
617-735-6791
fax 735-7043



Harvard Medical School

Office of Technology Licensing &
Industry-Sponsored Research



333 Longwood Avenue, Suite 640
Boston, Massachusetts 02115

Telephone: 617-432-0920
Facsimile: 617-432-2788

Mr. Isao Takeda
Senior Manager, Licensing
Patent and Licensing Department
Takeda Chemical Industries, Ltd.
3-6, Doshomachi 2-chome
Chuo-ku, Osaka 541
JAPAN

Dear Mr. Takeda:

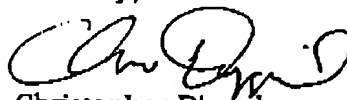
At the meeting, Dr. Folkman described the identification of previously unreported antiangiogenic properties of an known urinary metabolite of steroid metabolism, 2-methoxy-estradiol (2-MOE).

Upon returning to Boston, Dr. Folkman asked a postdoctoral fellow, Dr. Robert D'Amato to initiate the screening of 2-MOE for activity. Dr. D'Amato found that 2-MOE inhibits the growth of vessels in the CAM assay but not in the rabbit eye assay. In addition, he tested the compound in mouse against Lewis lung carcinoma and estimated a T/C of .35. In culture, 2-MOE was equally effective in preventing the growth of Lewis lung and endothelial cells, which suggests to Dr. D'Amato that



2-MOE is an antimitotic agent. Additionally, he has demonstrated that it inhibits microtubule formation similar to vinblastine, a current anticancer agent. When tested in vitro with AGM-1470, 2-MOE's effect was slightly increased. At this time, the results of these experiments have not been written up and Dr. D'Amato is now involved in other projects in addition to this one.

Sincerely,



Christopher Dippel
Project Manager

cc: Ms. Joyce Brinton
Mr. David Conlin
✓ Dr. Robert D'Amato
Mr. William New
Dr. Judah Folkman
Ms. Carol Quilty